THE FLAVONES OF RHUS*

CHAS. E. SANDO AND H. H. BARTLETT.

Some time ago our interest in the possible formation of anthocyanins from flavones in plants led us to investigate *Rhus glabra* L., *R. typhina* L., and *R. copallina* L., all of them species with yellow wood, red fruits and exhibiting exceedingly brilliant red autumnal coloring. From various species of Rhus investigated by Perkin,[1, 2] flavones have been isolated and identified. No great difficulty was therefore anticipated in the study of the flavones, and these have been isolated and identified from *Rhus glabra* (leaves, both green and red, wood, and berries), *Rhus typhina* (wood), and *Rhus copallina* (green leaves). We have not been successful thus far in isolating the red pigments of the berries and autumn leaves, but the distribution of the flavones themselves seems sufficiently interesting to justify the publication of a note on the subject.

The genus Rhus, in the broad sense, includes several subdivisions which really seem to merit recognition as genera. The type species of Rhus in the restricted sense is, as Greene[3] has shown, *Rhus Coriaria* L., the Sicilian sumach. With this species, to which on account of its commercial importance most of the chemical investigators have directed their attention, the three species studied by us are congeneric. One of them, *Rhus glabra*, is a collective species, including a number of elementary species, but their limits are not well known, and for the purpose of this paper it will suffice to use the name *R. glabra*, with the qualification that the data apply only to a form occurring about Ann Arbor, Michigan.

* The work here reported was carried on in the laboratories of the office of fermentation and physiological investigations, Bureau of Plant Industry, and the Department of Botany of the University of Michigan. Published by permission of the Secretary of Agriculture.

[1] Perkin, A. G., and Allen, G. Y. Colouring matter of Sicilian sumach, *Rhus Coriaria*. Journ. Chem. Soc. (London), Trans. **69**: 1299–1303. 1896.

[2] Perkin, A. G. Yellow colouring principles contained in various tannin matters. Part VI. *Rhus Cotinus* and *Rhus Rhodanthema*. Journ. Chem. Soc. (London), Trans. **73**: 1016–1019. 1898.

[3] Greene, E. L. A study of *Rhus glabra*. Proc. Washington Acad. Sci. **8**: 167–196. 1906.

Perkin has isolated flavones from *Rhus Coriaria*,[4] *R. Metopium*[4] (= *Metopium Metopium* (L.) Small), *R. Cotinus*[2] (= *Cotinus Coggyria* Scop.), and *R. rhodanthema* F. v. M.[2] (= *Rhodosphaera rhodanthema* (F. v. M.) Engl.). Only in the last two does the wood contain a flavone, and in both cases it is fisetin. In the leaves of the first two, the leaf flavone is myricetin, in the third both myricetin and quercetin occur, and in the last quercetin[2] alone. Perkin[1] shows definitely that early workers who ascribed quercetin (either free or glucosidal) to the leaves of *R. Coriaria* and *R. Cotinus* were in error. The data given above provide our only reliable data in regard to the distribution of the flavones in Rhus. Perkin emphasizes the fact that in all species thus far investigated the wood flavone is different from the leaf flavone. Our paper provides further data for establishing a generalization with regard to this point, and also gives the first identifications of flavones from American sumachs.

Acree and Syme[5] have reported fisetin from the leaves of *Rhus Toxicodendron* L. (= *Toxicodendron vulgare* Mill.), in which they suppose it to occur both free and as one constituent of a glucoside to which they ascribe the poisonous properties of this plant. They give no analysis of the flavone which they isolated, but from their own statement that it yields protocatechuic acid and phloroglucinol on potash fusion, we infer that it was quercetin rather than fisetin. Fisetin would have given protocatechuic acid and resorcinol. In a recent refutation of a paper by McNair[6] in which the work of Acree and Syme is called into question, Acree[5] explicitly verifies the earlier statement of Acree and Syme that a quantity (two grams) of the flavone which they obtained from the leaves of *R. Toxicodendron* and identified as fisetin was decomposed by potash fusion into phloroglucinol and protocatechuic acid. He states that the color reactions of the flavone were those of fisetin, but unless these substances are very carefully purified, there is much likelihood of error. Quercetin or even luteolin

[4] Perkin, A. G. Yellow colouring principles contained in various tannin matters. Part VII. *Arctostaphylos Uva-ursi, Haematoxylon Campeachianum, Rhus Metopium, Myrica Gale, Coriaria myrtifolia,* and *Robinia pseudacacia.* Journ. Chem. Soc. (London), Trans. 77: 423–432. 1900.

[5] Acree, S. F., and Syme, W. A. Some constituents of the poison-ivy plant. Amer. Chem. Journ. 36: 301–321. 1906. The same paper under a different title. Journ. Biol. Chem. 2: 547–573. 1906–7.

[6] McNair, J. B. The poisonous principle of poison oak. Journ. Amer. Chem. Soc. 38: 1417–1421. 1916.

might have been confused with fisetin, and it is extremely likely that quercetin was the flavone which they obtained. Not only is it more widely distributed than luteolin, but it is already known from the leaves of *Rhus rhodanthema*.[2] Even on the supposition that Acree and Syme incorrectly identified the potash fusion products of their flavone, it is still unlikely that they had fisetin, since the evidence now at hand seems to show that the latter does not occur in leaves, even when present in wood of the same plant. In their papers Acree and Syme refer to early work of Schmid[7] who did indeed state (erroneously) that one of the products resulting from the potash fusion of fisetin was phloroglucinol. His work preceded the determination of the constitution of fisetin, and has been superseded.

McNair made gasoline extractions of *Rhus diversiloba* T. and G. (a species closely allied to *R. Toxicodendron*) and failed to find any flavone at all in the extracts. This result is, of course, what one would expect, since both the free flavones and their glucosides are insoluble in gasoline. McNair makes the point, which seems to be well taken, that since the poison of the poison-ivy is soluble in gasoline, whereas the flavone glucosides are not, Acree and Syme cannot have been correct in ascribing the poisonous qualities of the plant to such a glucoside. On the contrary, his negative work obviously has no bearing on the question of what flavones occur in the poison-ivy.

The Wood Pigment, Fisetin

The wood of *R. typhina* was collected near Washington, D. C. A single log, four feet long and about seven inches in diameter, afforded all the material used. The wood of *R. glabra* was all collected from a single thicket near Ann Arbor, Michigan. The maximum diameter of the sticks was about 2 inches. In the case of each species the method of extracting the flavone, which proved to be fisetin, was as follows. The white sap wood was removed, and the yellow heart wood reduced to small chips, which were boiled for several days with successive portions of distilled water. The decoctions were combined, evaporated to small bulk, filtered, and shaken out with ether. The impure ethereal solution of fisetin thus obtained was evaporated and the residue extracted repeatedly with water, in which fisetin is practically insoluble, whereas certain colored impurities are soluble. It was then

[7] Schmid, Jakob. Ueber das Fisetin, den Farbstoff des Fisetholzes. Ber. Deutsch. Chem. Ges. 19: 1734–1749. 1886.

dissolved in a small volume of hot alcohol, filtered, and fractionally precipitated with water. The fractions were separately dried and acetylated by heating for an hour with anhydrous sodium acetate and acetic anhydride. The reaction mixture was poured into water and after twenty-four hours the precipitated acetyl fisetin was filtered off and purified by recrystallization from alcohol. The acetyl derivative formed a mat of silk-like colorless needles, insoluble in water, insoluble in cold, and sparingly soluble in hot alcohol, and easily soluble in warm glacial acetic acid. Since the several fractions had the same melting point, 199–200.5° C. (uncorrected), they were combined and again purified by recrystallization. The purified acetyl fisetin, derived from *R. typhina*, gave a yield of 61.66 percent of fisetin by hydrolysis, agreeing satisfactorily with the theoretical yield of 62.99 percent from $C_{15}H_6O_6(C_2H_3O)_4$. Had the formula of the acetyl compound been $C_{15}H_5O_6(C_2H_3O)_5$ theory would have required 57.66 percent flavone; if $C_{15}H_7O_6(C_2H_3O)_3$, 69.41 percent. The hydrolysis was carried out in acetic acid solution, with sulphuric acid. The recovered flavone was precipitated by the addition of water. The acetyl fisetin derived from both species had the same melting point, and since combustions of the recovered flavone were made for both, it was deemed sufficient to make combustions of the acetyl fisetin from one source only. The results are given in Table I.

TABLE I

Combustions of Acetyl Derivative of Fisetin from Wood of Rhus typhina

Sample	Weight	CO_2	H_2O	C, %	H, %	O, %
A	.1225	.2724	.0418	60.64	3.82	35.54
B	.1054	.2372	.0382	61.37	4.06	34.57
C	.2046	.4600	.0724	61.31	3.96	34.73
Arithmetic mean of three determinations	.....	.....	.....	61.10	3.94	34.96
Weighted mean	.....	.....	.....	61.14	3.95	34.91
Required for $C_{15}H_6O_6(C_2H_3O)_4$	.....	.....	.....	60.79	3.96	35.25

The pure fisetin was of a pale lemon yellow color, insoluble in cold water, very sparingly soluble in hot water, readily in alcohol and acetone. It was removed from aqueous or very dilute alcoholic solutions by acetic ether or ether, but after drying dissolved in ether only with difficulty. It was insoluble in benzene and chloroform. With ferric chloride it gave an olive-green coloration, with lead acetate an orange-red precipitate, with ammonia and other alkalies an intensification of

the yellow color. The data for the combustions of fisetin follow in Table II.

TABLE II

Combustions of Fisetin, Recovered from Acetyl Fisetin

Sample *A* from wood of *Rhus typhina*, sample *B* from wood of *R. glabra*.

Sample	Weight	CO_2	H_2O	C,%	H,%	O,%
A	.1074	.2482	.0350	63.02	3.64	33.34
B	.2550	.5870	.0796	62.78	3.49	33 73
Required for fisetin, $C_{15}H_{10}O_6$	.....	.....	.....	62.94	3.49	33.57

The cleavage products of fisetin were determined in the usual way by potash fusion. The material was heated thirty minutes at 170–200° C. with potassium hydroxide and a very small amount of water. The melt was dissolved in water, neutralized with hydrochloric acid, and shaken with ether. The residue after evaporating the etherial solution was neutralized with sodium bicarbonate and again shaken with ether.

The ethereal layer contained resorcinol, identified as uch after purification by sublimation between watch glasses. It was easily soluble in water, gave a violet color with ferric chloride, and melted at 106–108° C. (uncorrected). Rosenthaler (Der Nachweis organischer Verbindungen) accepts the value 110–111°, but quotes 118° as E. Schmidt's determination of the melting point of the absolutely pure compound. In some of the text-books (*e. g.*, Richter) the melting point is given as 118°. Landolt-Börnstein gives it as 111.6°, and this value is undoubtedly correct, being taken from a recent determination (1911) of Timmermans. Perkin and Gunnell[8] found that a Kahlbaum preparation melted at 108–109° C. With this value ours is in excellent agreement.

The sodium carbonate solution, after the removal of the resorcinol, was acidified and shaken out with ether. The latter removed a substance shown to be protocatechuic acid. It decomposed on heating, yielding a sublimate of catechol, identified as such by its melting point and reaction with ferric chloride (a green color, passing to violet, then red, upon addition of sodium bicarbonate).

There can be no doubt, therefore, that fisetin is the wood flavone of both *Rhus typhina* and *R. glabra*.

[8] Perkin, A. G., and Gunnell, O. The colouring matter of Quebracho Colorado. Journ. Chem. Soc. (London), Trans. **69**: 1303–1309. 1896.

The Leaf Pigment, Myricetin

Several variations in method were used for the isolation of myricetin from the leaves. The best yield was obtained by the method of Perkin. This method consists in fractionally precipitating the dissolved substances from an aqueous extract of the leaves, with lead acetate. On the addition of this reagent impurities (tannins, gums, resins, etc.) are first precipitated as lead compounds and may be removed by filtration. The flavones and their glucosides are only precipitated upon the addition of an excess of lead acetate.

The dried leaf powder was treated several days with successive portions of boiling distilled water and the combined extracts evaporated to small bulk. Lead acetate was then cautiously added to the boiling mixture until a further quantity produced a yellowish precipitate. In this manner the impurities were got rid of, by filtering off the lead compounds first precipitated. Excess of lead acetate added to the filtrate produced an insoluble yellow lead salt of the flavone glucoside. This was filtered with suction, washed thoroughly with water and decomposed with boiling dilute sulphuric acid. Lead sulphate was filtered off, and the filtrate, when cold, shaken with ether. The residue after evaporation of the ether contained the flavone and gallic acid. The latter was removed by treatment with hot water. The flavone was filtered off, dried, and acetylated in the usual manner.

The other method that we found useful in the isolation of myricetin was as follows. The aqueous extract of the leaves was treated with a large amount of hide powder to remove tannin. The filtrate was then evaporated to small bulk and hydrolyzed with hydrochloric acid (33 percent by volume) for nearly an hour. An ether extract of the cold solution yielded the crude pigment, which was purified by the usual process of acetylization and recovery by hydrolysis.

Our yields of myricetin were very small, and insufficient for combustions to be made of the compound from all of the sources from which it was obtained. It was likewise impossible to make a potash fusion. Myricetin, however, has more characteristic qualitative reactions[9] than the other pigments of the flavone group. Ammonia and dilute alkalies give a green coloration, changing to blue, violet, and finally reddish-brown. No other known flavone gives this play

[9] Perkin, A. G., and Hummel, J. J. The colouring principle contained in the bark of *Myrica nagi*. Part I. Journ. Chem. Soc. (London), Trans. **69**: 1287–1294. 1896.

of colors. Ferric chloride gives a brownish-black color. The myricetin is darker in color than fisetin, and shows about the same solubilities; it differs in being slightly soluble in chloroform and only very slightly in acetic acid.

The largest sample of myricetin was obtained from leaves of *Rhus glabra*. The acetyl myricetin from this source yielded 55.00 percent of myricetin on hydrolysis. Theory requires 55.79 percent for $C_{15}H_4O_8(C_2H_3O)_6$, the formula of the compound. For $C_{15}H_5O_8(C_2H_3O)_5$ and $C_{15}H_3O_8(C_2H_3O)_7$ the yields would have been 60.22 percent and 51.96 percent respectively. Two combustions of the acetyl myricetin and one of myricetin were carried out, with results shown in Table III. The acetyl myricetin melted at 208–209° C. (uncorrected) when slowly heated. Perkin gives the melting point as 211–212° C.

TABLE III

Combustions of Myricetin from Green Leaves of Rhus glabra (Sample A) and of its Acetyl Derivative (Samples B and C)

Sample	Weight	CO_2	H_2O	C,%	H,%	O,%
A	.0800	.1656	.0290	56.45	4.06	39.49
Required for myricetin, $C_{15}H_{10}O_8$				56.60	3.14	40.26
B	.0960	.2012	.0360	57.15	4.20	38.65
C	.1332	.2784	.0510	57.01	4.27	38.72
Mean of two determinations				57.08	4.24	38.68
Required for acetyl myricetin, $C_{15}H_4O_8(C_2H_3O)_6$				56.84	3.86	39.20

Myricetin both glucosidal, and, in very slight traces, free, was also obtained from the red autumn leaves of *R. glabra*, and from the red berries. In the latter case the myricetin was free, but it may have been derived by hydrolysis from a glucoside, since the berries when boiled in water yield a strongly acid solution. Two samples of green leaves of *Rhus copallina*, kindly furnished by Dr. W. W. Stockberger, of the Bureau of Plant Industry, also proved to contain myricetin. They were collected by C. R. Gilmore at Muskogee, Oklahoma. Several attempts to isolate a flavone from the leaves of *R. typhina*, collected at Ann Arbor, were unsuccessful, although various methods were used and the operations were conducted on a large scale.

SUMMARY

By the isolation of flavone pigments from three species of Rhus, *R. typhina*, *R. glabra*, and *R. copallina*, we have been able to verify

Perkin's conclusion that the same flavone is not likely to be found in both the wood and leaves of the same species. Fisetin is distinctively a wood flavone, and would appear to be an end product of metabolism. It is now known from *Rhus Cotinus*, *R. rhodanthema*, *R. typhina*, and *R. glabra*. The first two do not belong to Rhus in the restricted sense, but to the genera Cotinus and Rhodosphaera, respectively. Our studies are therefore the first to demonstrate the presence of fisetin in wood of species belonging to Rhus proper (the true sumachs).

The distinctive leaf flavone of Rhus proper is myricetin. It has been known from *R. Coriaria*, and we are able to add *R. glabra* and *R. copallina*. It is probably a plastic substance. Although we have thus far been unable to trace its relationship to the fisetin of the stem, or to the anthocyanins of the leaf and berries, efforts along this line will not be abandoned. The flavones are becoming increasingly interesting to the physiologist and geneticist, and on this account we venture to present this slight addition to our knowledge of their distribution in plants.